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1 1. A method of preparing a nucleic acid library, said method comprising introducing at least  
2 two members of an initial population of nucleic acid molecules into at least one cell, said  
3 population of nucleic acid molecules comprising two or more individual nucleic acids each of  
4 which consists of a nucleic acid sequence that is identical for each molecule and that includes an  
5 origin of replication; and a nucleic acid sequence that varies between members of said population  
6 and which comprises a substrate for recombination, said introducing resulting in recombination  
7 of said substrate for recombination between at least two members of the population thereby  
8 producing a population comprising recombined nucleic acid members.

1 2. The method of claim 1, wherein said recombination is performed by a recombination  
2 mechanism endogenous to said cell.

1 3. The method of claim 1, wherein said recombination is mediated by an exogenous  
2 recombinase.

1 4. The method of claim 1, wherein said recombination is mediated by an endogenous  
2 recombinase.

1 5. The method of claim 1, wherein said recombination is at a site preselected for  
2 recombination.

1 6. The method of claim 5, wherein the site preselected for recombination is a recombinase  
2 recognition site and the recombination is mediated by a recombinase expressed by said cell.

1 7. The method of claim 1, wherein said recombination is mediated by a recombinase  
2 selected from the group consisting of a member of the *hin* family of recombinases, a member of  
3 the *lambda* integrase family, an *flp* recombinase, a resolvase, a transposon, and a *Cre*  
4 recombinase.

1 8. The method of claim 7, wherein said recombinase is selected from the group consisting  
2 of *Cre*, *hin*, *gin*, *pin*, *cm*, and *flp*.

1 9. The method of claim 5, wherein said site preselected for recombination is a *loxP* site.

1 10. The method of claim 1, wherein said substrate for recombination, comprises a first site  
2 recombinase recognition site and a second recombinase recognition site different from the first  
3 recombinase recognition site.

1 11. The method of claim 10, wherein recombination results in the exchange, between two  
2 members of said nucleic acid population, of the nucleic acid flanked by the first and second  
3 recombinase recognition sites.

1 12. The method of claim 10, wherein the first recombinase recognition site is a loxP site and  
2 the second recombinase recognition site is a loxP mutant site.

1 13. The method of claim 12, wherein the loxP mutant site is loxP 511.

1 14. The method of claim 1, wherein said cell is selected from the group consisting of a  
2 bacterial cell, a yeast cell, an insect cell, and a mammalian cell.

1 15. The method of claim 14, wherein said bacterial cell is an *Escherichia coli* cell.

1 16. The method of claim 1, wherein said members of a population of nucleic acid molecules  
2 are introduced into the cell by transfection.

1 17. The method of claim 1, wherein said population of nucleic acid molecules comprises at  
2 least 10 different members.

1 18. The method of claims 1, wherein said members of a population of nucleic acid is  
2 molecules are contained within infectious particles and are introduced into the cells via infection  
3 with said infectious particles.

1 19. The method of claim 18, wherein said infectious particles are phage.

1 20. The method of claim 19, wherein said infectious particles are filamentous phage.

1 21. The method of claim 20, wherein the infectious particles are filamentous phage of the Ff  
2 family.

1 22. The method of claim 18, wherein said infectious particles are phagemids containing  
2 phagemidic DNA.

1 23. The method of claim 18, wherein said infectious particles are phagemids derived from  
2 filamentous phage of the Ff family.

1 24. The method of claim 1, wherein said method further comprises:  
2 transfecting or infecting one or more cells with members of said population of recombined  
3 nucleic acid members such that said cells are infected at a multiplicity of infection (moi) of less  
4 than about 1.

1 25. The method of claim 24, wherein said further method comprises the packaging the  
2 members of said nucleic acid library in replicable genetic display packages such that a protein on  
3 the surface of the replicable display package is encoded by a nucleic acid packaged within the  
4 display package that is a nucleic acid sequence that varies between members of the nucleic acid  
5 library.

1 26. The method of claims 1, wherein the variable nucleic acid sequence comprising the  
2 substrate for recombination comprises an expression cassette.

1 27. The method of claim 26, wherein said expression cassette comprises nucleic acid  
2 sequences encoding one or more polypeptides.

1 28. The method of claim 26, wherein said expression cassette comprises nucleic acid  
2 sequences encoding one or more polypeptides and the nucleic acid encoding at least one of said  
3 polypeptides is flanked by pair of recombinase recognition sites.

1 29. The method of claim 27, wherein said polypeptides are expressed on the surface of a  
2 phage, a phagemid, or a bacterium.

1 30. The method of claim 27, wherein said variable sequence includes nucleic acid encoding a  
2 first polypeptide chain and a second polypeptide chain from a specific binding pair member such  
3 that following recombination said variable sequence encodes binding proteins that are not  
4 present in the initial population of nucleic acids.

1 31. The method of claim 30, wherein said first and said second polypeptide are antibody  
2 polypeptides.

1 32. The method of claim 31, wherein said first and second polypeptide are selected from the  
2 group consisting of a V<sub>H</sub> region, a V<sub>L</sub> region, a V<sub>H</sub> CDR1, a V<sub>H</sub> CDR<sub>2</sub>, a V<sub>H</sub> CDR<sub>3</sub>, a V<sub>L</sub> CDR1,  
3 a V<sub>L</sub> CDR<sub>2</sub>, a V<sub>L</sub> CDR<sub>3</sub>, a V<sub>H</sub> joined to a C<sub>H</sub>1, and a V<sub>L</sub> joined to a C<sub>L</sub>.

1 33. The method of claim 32, wherein the first polypeptide is a V<sub>H</sub> region and the second  
2 polypeptide is a V<sub>L</sub> region.

1 34. The method of claim 30, wherein a pair of recombinase recognition sites flank the nucleic  
2 acid encoding a first polypeptide and said pair of recombinase recognition sites comprise a first  
3 recombinase recognition site and a different second recombinase recognition site.

1 35. The method of claim 34, wherein the first recombinase recognition site is a LoxP site and  
2 the second recombinase recognition site is a LoxP 511 site.

1 36. The method of claim 30, wherein the first polypeptide is flanked by a pair of recombinase  
2 recognition sites and the recognition sites are different from each other.

1 37. The method of claim 30, wherein the first polypeptide and the second polypeptide are  
2 each flanked by a pair of recombinase recognition sites and the recognition sites within each pair  
3 are different from each other.

1 38. The method of claim 36, wherein said loxP sites are selected from the group consisting of  
2 loxP, loxP 511, and fas loxP.

1 39. The method of claim 29, wherein the members of said library encode a single-chain  
2 antibody.

1 40. The method of claim 39, in which said antibody fragments are scFv.

1 41. The method of claim 29, wherein the members of said library encode a moiety selected  
2 from the group consisting of a Fab, an Fv, a diabody, a V<sub>H</sub> dimer, and a V<sub>L</sub> dimer.

1 42. The method of claim 29, wherein the members of said library encode an antibody in  
2 which the antibody V regions are linked by a polypeptide linker comprising a recombinase  
3 recognition site.

1 43. The method of claim 42, wherein said recombinase recognition site is selected from the  
2 group consisting of loxP, a loxP mutant, a recognition site for a hin family recombinase, a  
3 recognition site for a lambda integrase, recognition site for an flp recombinase, a recognition site  
4 for a resolvase, and a recognition site for a transposon.

1 44. A method according to claim 1, wherein the variable portion of the nucleic acid further  
2 comprises a selectable marker whereby said selectable marker must be recombined with a second  
3 selectable marker to become active.

1 45. The method of claim 44 wherein said selectable marker is inactive without recombination  
2 because of mutations.

1 46. The method of claim 44 wherein said selectable marker is inactive without recombination  
2 because it is an incomplete selectable marker.

1 47. The method of claim 44 wherein said selectable marker is located such that it is linked to  
2 the recombination substrate and co-transferred with a gene of interest in said recombination  
3 substrate.

1 48. A nucleic acid library made according to the method of claim 1.

1 49. A nucleic acid library comprising a population of nucleic acid molecules comprising two  
2 or more individual nucleic acids each of which consists of a nucleic acid sequence that is  
3 identical for each molecule and that includes an origin of replication and at least two  
4 recombinase recognition sites; and a nucleic acid sequence that varies between members of  
5 said population wherein said nucleic acid sequence that varies comprises a substrate for  
6 recombination, and wherein every member of said library has the same origin of replication.

1 50. The nucleic acid library of claim 49, wherein said library comprises at least 10 different  
2 members in a single cell.

1 51. The nucleic acid library of claim 49, wherein said library comprises at least 100 different  
2 members in a single cell.

1 52. The nucleic acid library of claim 49, wherein said recombinase recognition sites comprise  
2 sites recognized by a recombinase selected from the group consisting of a member of the hin  
3 family of recombinases, a member of the lambda integrase family, an flp recombinase, a  
4 resolvase, a transposon, and a Cre recombinase.

1 53. The nucleic acid library of claim 52, wherein said recombinase is selected from the group  
2 consisting of Cre, hin, gin, pin, cin, and flp.

1 54. The nucleic acid library of claim 49, wherein said recombinase recognition site is a LoxP  
2 site or a mutant LoxP site.

1 55. The nucleic acid library of claim 49, wherein said members of a population of nucleic  
2 acid molecules are contained within infectious particles.

1 56. The nucleic acid library of claim 55, wherein said infectious particles are phage.

1 57. The nucleic acid library of claim 55, wherein said infectious particles are filamentous  
2 phage.

1 58. The nucleic acid library of claim 55, wherein said infectious particles are filamentous  
2 phages of the Ff family.

1 59. The nucleic acid library of claim 55, wherein said infectious particles are phagemid  
2 containing phagemidic DNA.

1 60. The nucleic acid library of claim 55, wherein said infectious particles are phagemids  
2 derived from filamentous phage of the Ff family.

1 61. The nucleic acid library of claim 49, wherein the variable nucleic acid sequence  
2 comprising the substrate for recombination comprises an expression cassette.

1 62. The nucleic acid library of claim 61, wherein said expression cassette comprises nucleic  
2 acid sequences encoding one or more polypeptides.

1 63. The nucleic acid library of claim 62, wherein said polypeptides are expressed on the  
2 surface of a phage or phagemid.

1 64. The nucleic acid library of claim 49, wherein said variable sequence includes nucleic acid  
2 encoding a first polypeptide chain and a second polypeptide chain from a specific binding pair  
3 member such that following recombination binding nucleic acids encoding binding proteins are  
4 produced that are not present in the initial population of nucleic acids.

1 65. The nucleic acid library of claim 64, wherein said first and said second polypeptide are  
2 antibody polypeptides.

1 66. The nucleic acid library of claim 65, wherein said first and second polypeptide are  
2 selected from the group consisting of a  $V_H$  region, a  $V_L$  region, a  $V_H$  CDR1, a  $V_H$  CDR2, a  $V_H$   
3 CDR3, a  $V_L$  CDR1, a  $V_L$  CDR2, a  $V_L$  CDR3, a  $V_H$  joined to a  $C_H1$ , and a  $V_L$  joined to a  $C_L$ .

1 67. The nucleic acid library of claim 65, wherein the first polypeptide is a  $V_H$  region and the  
2 second polypeptide is a  $V_L$  region.

1 68. The nucleic acid library of claim 64, wherein a pair of recombinase recognition sites  
2 flank the nucleic acid encoding a first polypeptide and pair of recombinase recognition sites  
3 comprise a first recombinase recognition site and a different second recombinase recognition  
4 site.

1 69. The nucleic acid library of claim 68, wherein the first recombinase recognition site is a  
2 LoxP site and the second recombinase recognition site is a LoxP 511 site.

1 70. The nucleic acid library of claim 64, wherein the members of said library encode a  
2 single-chain antibody.

1 71. The nucleic acid library of claim 64, wherein the first and the second polypeptide are  
2 expressed on the surface of a phage or bacterium.

1 72. The nucleic acid library of claim 70, in which said polypeptides are scFv, in which V<sub>H</sub>  
2 and V<sub>L</sub> are joined by a polypeptide linker encoded by a nucleic acid comprising a loxP, a loxP  
3 mutant, a recognition site for a hin family recombinase, a recognition site for a lambda integrase,  
4 recognition site for an flp recombinase, a recognition site for a resolvase, and a recognition site  
5 for a transposon.

1 73. The nucleic acid library of claim 49, wherein said nucleic acids express rgdp  
2 polypeptides.

1 74. A method of preparing a polypeptide said method comprising:  
2 a) providing a nucleic acid library of claim 48;  
3 b) selecting one or more members of said library; and  
4 c) expressing the nucleic acids of the one or more selected members.

1 75. The method of claim 74, wherein said selecting comprises:  
2 i) expressing proteins encoded by the members of said nucleic acid library; and  
3 ii) screening the expressed proteins for one or more properties selected from the group  
4 consisting of specific binding to one or more preselected targets, a minimum binding avidity for  
5 one or more preselected targets, a maximum binding avidity for one or more preselected targets,  
6 thermostability at a particular preselected temperature, a predefined catalytic activity, a  
7 predefined enzymatic activity under selected conditions, a predefined biological activity; and  
8 iii) selecting the library members that meet the screening criteria.

1 76. The method of claim 75, wherein said screening comprises screening for specific binding  
2 to a preselected target.

1 77. The method of claim 75, wherein the expressed proteins comprise single chain  
2 antibodies.

1 78. The method of claim 75, wherein the polypeptides are expressed on the surface of cells  
2 infected or transfected with the members of the nucleic acid library.

1 79. The method of claim 75, wherein the polypeptides are expressed on the surface of  
2 replicable genetic display packages (rgdp).



1 79. The method of claim 75, wherein the polypeptides are expressed on the surface of  
2 replicable genetic display packages (rgdp).

1 80. The method of claim 75 where said members selected in step (iii) are used to enrich or  
2 generate a library according to the method of claim 1.

1 81. A procedure according to claim 79, in which said replicable genetic display package  
2 (rgdp) is a phagemid expressing one or more polypeptides bound to surface proteins.

1 82. A polypeptide encoded by a member of a nucleic acid library of claim 48.

1 83. A host cell comprising a nucleic acid library according to claim 48.

1 84. The host cell of claim 83, wherein said cell is selected from the group consisting of a  
2 bacterial cell, a plant cell, a mammalian cell, an insect cell, and a yeast cell.

1 85. A vector encoding a single chain antibody, where a nucleic acid encoding a fragment of  
2 said antibody is flanked by a pair of recombinase recognition sites where said recombinase  
3 recognition sites are different such that the nucleic acid encoding the fragment of the antibody  
4 can be exchanged between different plasmids of the same type via the action of a recombinase.

1 86. The vector of claim 85, wherein said antibody fragment is selected from the group  
2 consisting of a  $V_H$  region, a  $V_L$  region, a  $V_H$  CDR1, a  $V_H$  CDR2, a  $V_H$  CDR3, a  $V_L$  CDR1, a  $V_L$   
3 CDR2, a  $V_L$  CDR3, a  $V_H$  joined to a  $C_H1$ , and a  $V_L$  joined to a  $C_L$ .

1 87. The vector of claim 85, wherein said recombinase recognition sites are is selected from  
2 the group consisting of loxP, a loxP mutant, a recognition site for a *hin* family recombinase, a  
3 recognition site for a lambda integrase, recognition site for an *flp* recombinase, a recognition site  
4 for a resolvase, and a recognition site for a transposon.

1 88. The vector of claim 85, wherein said vector encodes an antibody comprising at least two  
2 antibody regions linked by a polypeptide linker comprising a recombinase recognition site.

1 89. The vector of claim 88, wherein said vector encodes an antibody in which the  $V_H$  and  $V_L$   
2 regions are linked by a polypeptide linker comprising a recombinase recognition site.

- 1 90. The vector of claim 89, wherein said vector is pDAN5.
- 1 91. A kit comprising a container containing a vector of claim 85.
- 1 92. A kit comprising a container containing members of the nucleic acid library of claim 49.